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=> S (OmpT (w) (protease or proteinase or peptidase)) or (Yersinia pestis plasminogen activator) or (Salmonella typhimurium E protein) or (Shigella flexneri SopA) or (omptin) or (ompp) or (coli (6A) protease VII)
L1 375 (OMPT (W) (PROTEASE OR PROTEINASE OR PEPTIDASE)) OR (YERSINIA PESTIS PLASMINOGEN ACTIVATOR) OR (SALMONELLA TYPHIMURIUM E PROTEIN) OR (SHIGELLA FLEXNERI SOPA) OR (OMPTIN) OR (OMPP) OR (COLI (6A) PROTEASE VII)

=> S 97 (6A) (met or methionine)
L2 489 97 (6A) (MET OR METHIONINE)

=> s 11 and 12
L3 1 L1 AND L2

=> d 13 bib ab

1.3 ANSWER 1

DN 142:368184

TI Production of biol

11 Production synthesis

synthetic polypeptide precursors by the Omp1 protease variants

PA Daiichi Suntory Pharma Co., Ltd., Japan
SO PCT Int. Appl., 107 pp.
CODEN: PIXXD2

DT Patent

LA Japanese

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2005030956	A1	20050407	WO 2004-JP14704	20040929
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
	RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	AU 2004276687	A1	20050407	AU 2004-276687	20040929
	CA 2540446	A1	20050407	CA 2004-2540446	20040929
	EP 1674567	A1	20060628	EP 2004-773628	20040929
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, FI, RO, CY, TR, BG, CZ, EE, HU, PL, SK				
	BR 2004014611	A	20061107	BR 2004-14611	20040929
	CN 1860226	A	20061108	CN 2004-80028525	20040929
	KR 2006089724	A	20060809	KR 2006-705984	20060327
	US 20070077617	A1	20070405	US 2006-573821	20060328
PRAI	JP 2003-342183	A	20030930		
	WO 2004-JP14704	W	20040929		

AB The proteolytic method for producing biol. active polypeptides (ACTH (1-24), motilin or calcitonin) from recombinant synthetic precursor polypeptides or fusion proteins by using OmpT protease mutants has been developed. The synthetic precursor polypeptides or fusion proteins (22 .apprx. 45 a.a. (amino acid)) have been designed according to the substrate specificities of the OmpT protease mutants. Synthetic substrate polypeptides have Arg or Lys at P1 site and the a.a. other than Asp, Glu or Pro at the P1' site. The substrate polypeptides have one, two or serial three basic a.a. in the P10 .apprx. P3, P10 .apprx. P3' or P10 .apprx. P5' (more specifically in the P5 .apprx. P3 site), however the sites P6 and P4 are excluded if only one basic a.a. in the sequence. The fusion protein substrates with protection peptide having C-terminal Arg or Lys have N-terminal a.a. such as Phe, Ala, Ser, Cys or Tyr and the other a.a. excluding Asp, Glu and Pro. These preferred P5 .apprx. P1 sequence and P7 .apprx. P1 sequence in the synthetic precursor polypeptides or fusion proteins are Arg-Arg-Arg-Ala-Arg and Asp-Ala-Arg-Arg-Ala-Arg, resp. Introduction of acidic a.a. typically Asp to the P3 site can repress the digestion by the OmpT proteases. The OmpT protease variants that can be used in the proteolysis system have a.a variation at the 97th position. The 97th a.a. is Leu, Met or His and the other a.a. including Ala, Phe, Ser, Thr, Cys, Asn, Gln, and Glu. The vector encoding the fusion substrate protein containing human glucagon, motilin, ACTH or calcitonin was designed to satisfy the structural condition claimed above and expressed in the inclusion body of E. coli and the cleaving of biol. active peptides from the substrate fusion proteins by the recombinant OmpT protease variant was demonstrated. The performance of the coexpression system of the substrate fusion protein and OmpT protease variant in the biol. active peptide generation was also demonstrated.

RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> S 97 near6 (variant or mutant or mutated or mutation or mutating or mutagenesis or substitution or substitute or substituted or replace or replaced or replacing)
MISSING OPERATOR 'NEAR6 (VARIANT'

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=> S 97 (6A) (variant or mutant or mutated or mutation or mutating or mutagenesis or substitution or substitute or substituted or replace or replaced or replacing)

L4 1768 97 (6A) (VARIANT OR MUTANT OR MUTATED OR MUTATION OR MUTATING
OR MUTAGENESIS OR SUBSTITUTION OR SUBSTITUTE OR SUBSTITUTED OR
REPLACE OR REPLACED OR REPLACING)

=> s 11 and 14

L5 2 L1 AND L4

=> d 15 1-2 bib ab

L5 ANSWER 1 OF 2 MEDLINE on STN

AN 2004028684 MEDLINE

DN PubMed ID: 14711628

TI Utilization of *Escherichia coli* outer-membrane endoprotease OmpT variants as processing enzymes for production of peptides from designer fusion proteins.

AU Okuno Kazuaki; Yabuta Masayuki; Ooi Toshihiko; Kinoshita Shinichi

CS Institute for Medicinal Research and Development, Daiichi Suntory Pharma Co., Ltd., Akaiwa, Chiyoda-machi, Ohra-gun, Gunma 370-0503, Japan..

Kazuaki_Okuno@dsup.co.jp

SO Applied and environmental microbiology, (2004 Jan) Vol. 70, No. 1, pp. 76-86.

Journal code: 7605801. ISSN: 0099-2240.

Report No.: NLM-PMC321264.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200404

ED Entered STN: 21 Jan 2004

Last Updated on STN: 9 Apr 2004

Entered Medline: 8 Apr 2004

AB *Escherichia coli* outer-membrane endoprotease OmpT has suitable properties for processing fusion proteins to produce peptides and proteins. However, utilization of this protease for such production has been restricted due to its generally low cleavage efficiency at Arg (or Lys)-Xaa, where Xaa is a nonbasic N-terminal amino acid of a target polypeptide. The objective of this study was to generate a specific and efficient OmpT protease and to utilize it as a processing enzyme for producing various peptides and proteins by converting its substrate specificity. Since OmpT Asp(97) is proposed to interact with the P1' amino acid of its substrates, OmpT variants with variations at Asp(97) were constructed by replacing this amino acid with 19 natural amino acids to alter the cleavage specificity at Arg (P1)-Xaa (P1'). The variant OmpT that had a methionine at this position, but not the wild-type OmpT, efficiently cleaved a fusion protein containing the amino acid sequence -Arg-Arg-Arg-Ala-Arg downward arrow motilin, in which motilin is a model peptide with a phenylalanine at the N terminus. The OmpT variants with leucine and histidine at position 97 were useful in releasing human adrenocorticotrophic hormone (1-24) (serine at the N terminus) and human

calcitonin precursor (cysteine at the N terminus), respectively, from fusion proteins. Motilin was produced by this method and was purified up to 99.0% by two chromatographic steps; the yield was 160 mg/liter of culture. Our novel method in which the OmpT variants are used could be employed for production of various peptides and proteins.

L5 ANSWER 2 OF 2 EMBASE COPYRIGHT (c) 2009 Elsevier B.V. All rights reserved on STN
AN 2004033574 EMBASE
TI Utilization of Escherichia coli Outer-Membrane Endoprotease OmpT Variants as Processing Enzymes for Production of Peptides from Designer Fusion Proteins.
AU Okuno, Kazuaki (correspondence); Yabuta, Masayuki
CS Inst. for Med. Res. and Development, Daiichi Suntory Pharma Co., Ltd., 2716-1 Kurakake, Akaiwa, Ohra-gun, Gunma 370-0503, Japan. Kazuaki_Okuno@dsup.co.jp
AU Okuno, Kazuaki (correspondence); Ooi, Toshihiko; Kinoshita, Shinichi
CS Division of Molecular Chemistry, Graduate School of Engineering, Hokkaido University, Sapporo, Hokkaido 060-8628, Japan. Kazuaki_Okuno@dsup.co.jp
SO Applied and Environmental Microbiology, (Jan 2004) Vol. 70, No. 1, pp. 76-86.
Refs: 30
ISSN: 0099-2240 CODEN: AEMIDF
CY United States
DT Journal; Article
FS 004 Microbiology: Bacteriology, Mycology, Parasitology and Virology
LA English
SL English
ED Entered STN: 12 Feb 2004
Last Updated on STN: 12 Feb 2004
AB Escherichia coli outer-membrane endoprotease OmpT has suitable properties for processing fusion proteins to produce peptides and proteins. However, utilization of this protease for such production has been restricted due to its generally low cleavage efficiency at Arg (or Lys)-Xaa, where Xaa is a nonbasic N-terminal amino acid of a target polypeptide. The objective of this study was to generate a specific and efficient OmpT protease and to utilize it as a processing enzyme for producing various peptides and proteins by converting its substrate specificity. Since OmpT Asp(97) is proposed to interact with the P1' amino acid of its substrates, OmpT variants with variations at Asp (97) were constructed by replacing this amino acid with 19 natural amino acids to alter the cleavage specificity at Arg (P1)-Xaa (P1'). The variant OmpT that had a methionine at this position, but not the wild-type OmpT, efficiently cleaved a fusion protein containing the amino acid sequence -Arg-Arg-Arg-Ala-Arg ↓ motilin, in which motilin is a model peptide with a phenylalanine at the N terminus. The OmpT variants with leucine and histidine at position 97 were useful in releasing human adrenocorticotrophic hormone (1-24) (serine at the N terminus) and human calcitonin precursor (cysteine at the N terminus), respectively, from fusion proteins. Motilin was produced by this method and was purified up to 99.0% by two chromatographic steps; the yield was 160 mg/liter of culture. Our novel method in which the OmpT variants are used could be employed for production of various peptides and proteins.

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